

Facilitation of iontophoretic drug delivery through intact and caries-affected dentine

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Abstract

Puapichartdumrong P, Ikeda H, Suda H. Facilitation of iontophoretic drug delivery through intact and caries-affected dentine. *International Endodontic Journal*, **36**, 674–681, 2003.

Aim To investigate the facilitative role of iontophoresis in drug delivery through intact and caries-affected dentine into the pulp.

Methodology Forty-eight intact dentine or caries-affected dentine discs were prepared from freshly extracted human third molars. The hydraulic conductance was measured before and after the experiments. Drug diffusion with and without iontophoresis (0.05 mA, 10 min) was evaluated using a split-chamber device. The enamel side chamber was filled with metronidazole (MN), sodium salicylate (SS) or naproxen sodium (NA), while the pulpal side was circulated with phosphate buffered saline (PBS) and pressurized (15 cmH₂O). Samples were collected after 1 h. Drug concentrations of the pulpal side solution were determined using a spectrophotometer. Then, electrical impedance of each dentine disc was measured. Finally, the dentine

disc surfaces were observed by scanning electron microscopy.

Results Drug diffusion was significantly influenced by iontophoresis, caries-induced changes in dentine and the drugs used (three-way ANOVA, $P < 0.05$). The diffusion of all the drugs through caries-affected dentine was significantly less than that through intact dentine (independent t -test, $P < 0.05$). Also, iontophoresis facilitated the diffusion of all the drugs through intact and caries-affected dentine. The drug diffusion of SS was significantly higher than MN and NA (one-way ANOVA, $P < 0.05$), independent of iontophoresis.

Conclusions Presence of dental caries may inhibit drug diffusion through dentine into the pulp. However, iontophoresis could enhance the delivery of ionized drugs through both intact and caries-affected dentine.

Keywords: caries-affected dentine, intact dentine, iontophoresis, metronidazole, naproxen sodium, sodium salicylate.

Received 31 January 2003; accepted 12 June 2003

Introduction

Whether pulpal inflammation is reversible or not is a matter of clinical importance. Some medicaments such as antibiotics, glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs) may save the pulp in the boundary zone between reversible and irreversible pulpitis (Ciarlone & Pashley 1992).

When a medicament is topically applied to dentine, the drug diffusion into the pulp is inhibited by an outward flow of dentinal fluid (Vongsavan & Matthews 1992). Additionally, dentine sclerosis or reparative dentine formation following physiological or pathological stimulation results in a reduction of dentine permeability (Stanley *et al.* 1983) and appears to influence the drug diffusion through dentinal tubules. Even if the drugs reach the pulpal tissue, odontoblasts (Pashley *et al.* 1978a) and pulpal microcirculation (Ciarlone & Pashley 1992) may prevent the drugs from reaching an effective concentration.

To overcome these problems, the use of more potent drugs or vasoconstrictors has been recommended (Ciarlone & Pashley 1992). A pulpward hydrostatic

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pressure against the outward pressure through dentinal tubules has been shown to effectively deliver lidocaine solution into the pulp *in vivo* (Ozawa *et al.* 2002). The process of passing a small electrical current through the tissue, iontophoresis, may be an alternative noninvasive method to deliver ionized drugs into the tooth pulp (Singh & Maibach 1994). Iontophoresis has been applied in dental treatment, such as pain relief from hypersensitive dentine (Carlo *et al.* 1982), aphthous ulceration (Gangarosa 1983) and anaesthesia of oral mucosa with lidocaine (Gangarosa 1974).

Iontophoresis of metronidazole (MN) anions has been evaluated using a paper electrophoresis technique (Kamath & Gangarosa 1995). Also, iontophoresis of salicylic acid and naproxen has been evaluated in rat skin (Tashiro *et al.* 2001). However, to what extent iontophoresis facilitates the diffusion of these drugs into the pulp, against an outward dentinal fluid flow, has not yet been clarified. Thus, the aim of the present study was to investigate the facilitative role of iontophoresis in drug delivery through intact and caries-affected dentine into the pulp.

Materials and methods

Preparation of drugs

Metronidazole, naproxen sodium (NA) and sodium salicylate (SS) (Sigma-Aldrich, MO, USA) solutions were adjusted to pH 7.4 using phosphate buffered solution, and then subsequently diluted into various concentrations. Standard graphs were drawn using a spectrophotometer (V-550, Jasco, Tokyo, Japan) to determine absorbance at 320, 231 and 296 nm for MN, NA and SS, respectively. For a drug diffusion test, 0.05 mol L⁻¹ solutions of each drug were prepared and the concentrations of the drugs that diffused through dentine discs were detected by spectrophotometry.

Dentine discs

A total of 48 human molars extracted for clinical reasons were used in the experiment. Donor patients (aged 29 ± 7 years) gave informed consent for their extracted teeth to be used in the study. Dentine discs were prepared from either dentine beneath a carious lesion without any sign of pulp exposure or intact dentine (24 discs for each group). Prior to use, the teeth were stored in 0.01 mol L⁻¹ phosphate buffered saline (PBS) at 4 °C for not more than 4 weeks. Carious dentine was removed using a low-speed round steel bur and a Caries Detector

(Kuraray, Osaka, Japan). The remaining dentine thickness between the cavity floor and the pulpal horn was not less than 0.7 mm. Using a low-speed diamond blade (Isomet[®], Buehler, IL, USA) under copious water cooling, the dentine discs were sliced as close as possible to the pulp chamber. The discs, measuring 0.70 ± 0.05 mm in thickness, were polished on abrasive paper (numbers 320, 500 and 1000). The smear layers on both sides were then removed with 10% phosphoric acid for 10 s. The surfaces were rinsed with tap water for 5 min and the discs were put in an ultrasonic cleaner for 10 min.

Hydraulic conductance measurement

Before and after the drug diffusion test, the hydraulic conductance (L_p) of each dentine disc was determined to evaluate dentine permeability. The dentine discs were randomly assigned to one of three drug groups. Double O-rings (6 mm in diameter) were inset in a split-chamber device (Outhwaite *et al.* 1974) and both the chambers (pulpal side and enamel side) were filled with PBS. The same hydrostatic pressure as intrapulpal pressure, that is, 15 cmH₂O (Vongsavan & Matthews 1992), was applied to the pulpal side of the chamber by raising the outflow tubing. PBS was then pumped into the pulpal side of the chamber at a rate of 0.2 mL min⁻¹ from a container (Matthews & Adrew 1995). A 50-μL microsyringe was connected to the enamel side of the chamber (Fig. 1), and the rate of fluid movement across the dentine disc was measured by monitoring the movement of a small air bubble (1 μL) in the microsyringe. To confirm the air tightness and to check the air bubble movement, a plastic disc or a 0.2-μm cellulose filter was inserted in place of the dentine disc.

Fluid flux was given by:

$$J_v = \frac{Q}{At}$$

where J_v (μL cm⁻² min⁻¹) is the fluid flux; Q (μL) is the fluid shift; A (cm²) is the area of dentine and t (min) is the diffusion time.

Hydraulic conductance was calculated as follows:

$$L_p = \frac{J_v}{P} \text{ (Pashley *et al.* 1996)}$$

Where L_p (μL cm⁻² min⁻¹ cmH₂O⁻¹) is the hydraulic conductance; J_v (μL cm⁻² min⁻¹) is the fluid flux and P (cmH₂O) is the applied hydrostatic pressure.

Drug diffusion test

The split-chamber device was set horizontally when the dentine disc was inserted for the hydraulic conductance

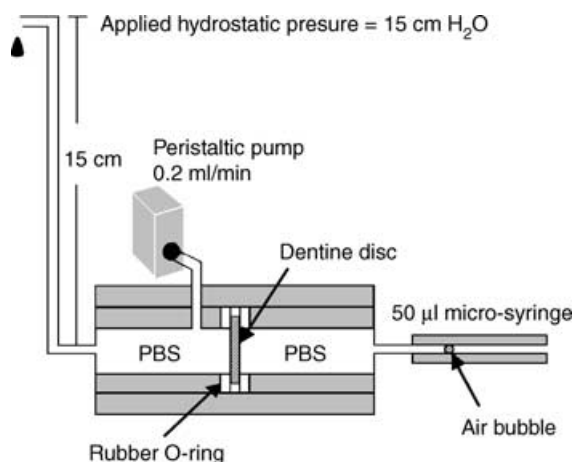


Figure 1 Diagram of the experimental system for hydraulic conductance measurement (PBS, phosphate buffered saline).

measurement. The enamel side of the chamber without the microsyringe was filled with one of the three drugs, whereas the pulpal side was circulated with PBS (0.2 mL min^{-1}). Outward pulpal pressure environment was simulated as high as $15 \text{ cmH}_2\text{O}$ by placing the out-flow tubing at a level of $15 \text{ cmH}_2\text{O}$ above the position of the dentine disc (Fig. 1). Thus, drug diffusion was tested in a passive way (control). For iontophoresis (MR-52 class 2.5, Onuki Medical Apparatuses, Tokyo, Japan) at 0.05 mA for 10 min , the platinum cathodal electrode with a flat end (0.5 mm diameter) was connected to the enamel side of the chamber and the anodal electrode to the pulpal side. Pulpal solution was sampled from the outflow of the tubing every 2 min for the first 10 min and every 5 min for the following 50 min . Drug concentrations were determined using a spectrophotometer. Drug diffusion tests were repeated with one dentine disc for the same drug, with and without iontophoresis.

Electrical impedance measurement

Electrical impedance of each dentine disc was measured using an impedance meter (3532 LCR HiTESTER, HIOKI, Nagano, Japan) at a frequency of $1, 8, 10$ and 100 kHz . The dentine disc was inserted in the split-chamber containing PBS. A platinum electrode (Inter Medical Co., Nagoya, Japan) (0.5 mm diameter) was connected to the enamel side of the chamber and an indifferent electrode to the pulpal side. As a control, a dentine disc was not set between O-rings.

Scanning electron microscopic observation

After the electrical impedance measurement, the surfaces of all dentine discs were observed under a scanning electron microscope (SEM; JSM-5310LV, JEOL, Tokyo, Japan).

Data analyses

The effects of iontophoresis, caries-induced changes in dentine and the drug type on the hydraulic conductance were analysed using the three-way ANOVA.

In the diffusion test, the amount of drug that diffused through dentine was determined by concentrations within the collected samples. The relation between concentration and time was plotted, and then the integrated area was calculated. The effects of iontophoresis, presence of caries and the drug type on drug diffusion were analysed by the three-way ANOVA. Finally, multiple comparisons were made to check the differences among the three drugs. Using the independent and the paired *t*-tests, drug concentrations of the intact and caries-affected dentine groups, and those of the passive and iontophoretic delivery groups were compared. Using the independent *t*-test, the electrical impedance of intact and caries-affected dentine was also compared. A probability value of $P < 0.05$ was considered as significant.

Results

The hydraulic conductance of each dentine disc was measured before and after the drug diffusion test, as shown in Table 1. Hydraulic conductance was not influenced by iontophoresis or the drugs used, but was influenced by presence of caries (three-way ANOVA, $P < 0.05$). The hydraulic conductance of intact dentine was higher than that of caries-affected dentine (independent *t*-test, $P < 0.05$).

Drug diffusions through intact and caries-affected dentine are shown in Figs 2–4. The integrated areas were calculated and compared among the experimental groups. Using three-way ANOVA, the drug diffusion was significantly influenced by iontophoresis, the presence of caries and the drug type ($P < 0.05$). In combination with iontophoresis, there was a significantly faster increase in the concentration of all drugs through both intact and caries-affected dentine compared to controls (paired *t*-test, $P < 0.05$). The change in drug concentration with time is shown graphically as a pulpal-drug concentration curve (Figs 2–4). First, drug concentration increased, suggesting that the rate of drug diffusion

Table 1 Influence of iontophoresis and presence of caries on L_p

Drugs	Hydraulic conductance (L_p) ($\times 10^{-2} \mu\text{L cm}^{-2} \text{min}^{-1} \text{cmH}_2\text{O}^{-1}$) (mean $\pm$ SD)					
	Intact dentine ^a			Caries-affected dentine ^b		
	Control ^c	Passive ^d	ION ^e	Control ^c	Passive ^d	ION ^e
MN	12.14 $\pm$ 5.47	11.44 $\pm$ 5.00	11.60 $\pm$ 5.44	2.78 $\pm$ 1.25	2.88 $\pm$ 1.04	3.03 $\pm$ 1.12
SS	10.14 $\pm$ 4.11	9.80 $\pm$ 4.20	9.52 $\pm$ 4.50	2.29 $\pm$ 1.20	2.32 $\pm$ 1.22	2.31 $\pm$ 1.04
NA	10.07 $\pm$ 3.61	10.07 $\pm$ 3.25	10.72 $\pm$ 4.32	1.96 $\pm$ 1.08	2.18 $\pm$ 1.50	2.10 $\pm$ 1.45

L_p was not influenced by iontophoresis but by the presence of caries in all drugs (three-way ANOVA, $P < 0.05$). ^a L_p of intact dentine was significantly higher than ^b L_p of caries-affected dentine (independent t -test, $P < 0.05$).

^c L_p before drug diffusion test.

^d L_p after passive drug delivery test.

^e L_p after iontophoretic drug delivery test.

was higher than the rate of elimination. In the present study, PBS was pumped to the pulpal chamber at a constant rate (0.2 mL min^{-1}) to simulate the drug elimination. After stopping iontophoresis, the concentration of drugs was still at a high level and then gradually fell to the same concentration level as that of the control. After 40 min, no significant difference of pulpal-drug concentration was found between iontophoretic and passive drug deliveries of all the drugs. The diffusion of all the drugs through caries-affected dentine was less than that through intact dentine (independent t -test, $P < 0.05$). The total amount of diffused drug through both intact and caries-affected dentine was significantly more in SS than in MN and NA (one-way ANOVA, $P < 0.05$), irrespective of whether iontophoresis was applied or not.

Caries-affected dentine showed higher electrical impedance values than intact dentine at all current frequencies measured (independent t -test, $P < 0.05$, Fig. 5).

The SEM observation of disc surfaces showed that the dentinal tubules of intact dentine were left open, while those of caries-affected dentine contained irregular mineral deposits in the dentinal tubules (Fig. 6).

Discussion

Facilitative role of iontophoresis in drug diffusion

This is the first report to demonstrate the facilitation of iontophoretic delivery of ionized drugs through dentine against an outward dentinal fluid flow. The drug

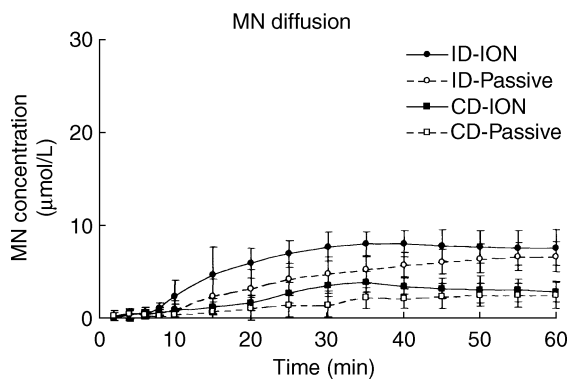


Figure 2 The facilitative effect of iontophoresis (ION) on MN diffusion through intact dentine (ID) and caries-affected dentine (CD). Iontophoresis and the presence of caries influenced the diffusion of all drugs (three-way ANOVA, $P < 0.05$). The drug diffusion through caries-affected dentine was less than that through intact dentine (independent t -test, $P < 0.05$). Iontophoresis facilitated the drug diffusion through intact and caries-affected dentine compared to control (paired t -test, $P < 0.05$).

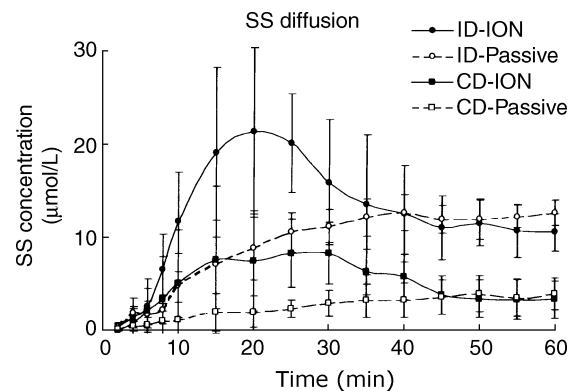


Figure 3 The facilitative effect of ION on SS diffusion through ID and CD. Iontophoresis and the presence of caries influenced the diffusion of all drugs (three-way ANOVA, $P < 0.05$). The drug diffusion through caries-affected dentine was less than that through intact dentine (independent t -test, $P < 0.05$). Iontophoresis facilitated the drug diffusion through intact and caries-affected dentine compared to control (paired t -test, $P < 0.05$).

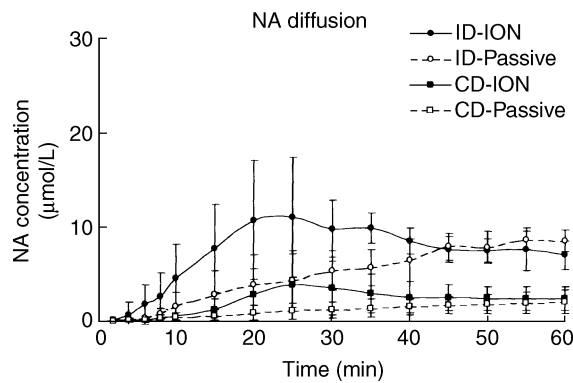


Figure 4 The facilitative effect of ION on NA diffusion through ID and CD. Iontophoresis and the presence of caries influenced the diffusion of all drugs (three-way ANOVA, $P < 0.05$). The drug diffusion through caries-affected dentine was less than that through intact dentine (independent t -test, $P < 0.05$). Iontophoresis facilitated the drug diffusion through intact and caries-affected dentine compared to control (paired t -test, $P < 0.05$).

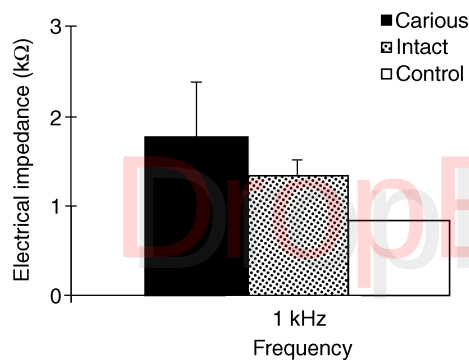


Figure 5 Electrical impedance of intact and caries-affected dentine. The electrical impedance of caries-affected dentine was significantly higher than that of intact dentine at 1, 8, 10 and 100 kHz (independent t -test, $P < 0.05$). Dentine disc was not set between O-rings in controls.

diffusion was increased by the effect of the electric current on repelling ionized drugs and the electrochemical potential gradient across the dentine disc (Singh & Maibach 1994). The hydraulic conductance measured after iontophoresis did not change significantly (Table 1), and the permeation of drugs gradually decreased to the same concentration level as the control after the cessation of iontophoresis (Figs 2–4). Thus, the drug diffusion was not increased by the alteration of dentine permeability itself, which was consistent with the previous study by Pashley *et al.* (1978b).

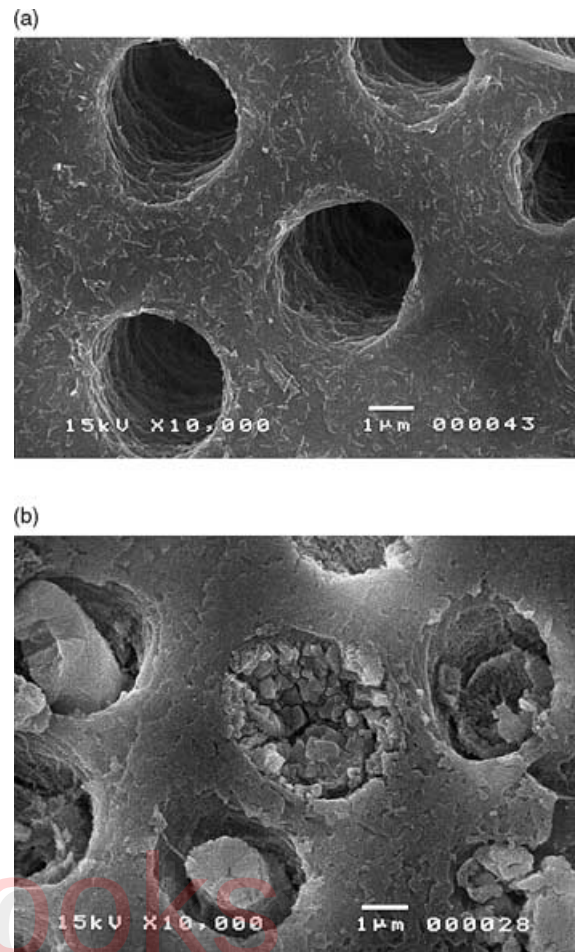


Figure 6 (a) Scanning electron micrographs of intact dentine surface showing opened dentinal tubules (10 000 $\times$, bar = 1 μ m). (b) Scanning electron micrographs of caries-affected dentine surface showing occluded dentinal tubules (10 000 $\times$, bar = 1 μ m).

Using iontophoresis, the three ionized drugs could easily pass through dentinal tubules, even in caries-affected dentine, which had a lower permeability and a higher electrical impedance than intact dentine (Figs 2–4). When a constant current source is used in iontophoresis, the voltage is increased to compensate for resistance increases during current delivery (Walker *et al.* 1995).

The present findings suggested that the application of ionized drugs in combination with iontophoresis could enhance the diffusion of drug through dentine against the outward dentinal fluid flow. This technique might be useful for a clinical practice, however, this has not been confirmed. If this technique is applicable to clinical

practice, it may be suitable to treat inflammatory pulps in which the clinical signs and symptoms of irreversible pulpitis are not distinguishable. In such cases, pulpal interstitial tissue pressure at inflamed areas is supposedly increased, which may result in an increase in outward dentinal fluid flow. Topical application of drugs such as antibiotics or NSAIDs with iontophoresis may facilitate the drug diffusion into the pulp and may raise the chance of pulpal healing.

Constant current (0.05 mA for 10 min; 0.5 mA min) was chosen in the present study on the grounds that an application of direct electric current to cervical dentine, up to 1 mA for 5 min (5 mA min) caused neither histological nor ultrastructural alterations in the dog tooth pulp (Walton *et al.* 1979). Additionally, a pilot study showed that the diffused MN through intact dentine at 0.3 and 0.5 mA min were current intensity-dependent. The current strength used in this study could cause pain *in vivo*. In such cases, anaesthetizing the tooth prior to iontophoresis would appear desirable.

Influence of dental caries on drug diffusion

The hydraulic conductance and diffusion of the three drugs through caries-affected dentine were less than those through intact dentine (Table 1; Figs 2–4). Additionally, the SEM images of caries-affected dentine surfaces showed mineral deposits in the dentinal tubules (Fig. 6b). The present results were consistent with previous studies showing that carious dentine is less permeable than intact dentine (Pashley *et al.* 1991). The decrease in dentine permeability owing to dentine sclerosis is one of the protective reactions of the pulp against caries (Kim *et al.* 2002). As a result of dentine sclerosis, the dentine permeability decreases and leads to the decrease in the hydraulic conductance and drug diffusion. Thus, the low permeability of caries-affected dentine might prevent drugs from reaching the therapeutic concentration in the pulp.

Other factors such as formation of reparative dentine (Stanley *et al.* 1983), presence of the odontoblasts, and pulp tissue (Pashley *et al.* 1978a) may also affect dentine permeability. During dentine disc preparation, it was difficult to evaluate whether reparative dentine remained or not. Therefore, it cannot be concluded that reparative dentine caused a reduction in dentine permeability, because the innermost dentine layer was removed during preparation of the smooth flat dentine discs. Furthermore, the experimental model did not include odontoblasts or pulp tissue. Hence, the present results might not be directly applicable to teeth *in vivo*.

Influence of drugs used

The integrated dose of diffused SS was about twice as much as that of MN and NA in both intact and caries-affected dentine, whether iontophoresis was applied or not (Figs 2–4). A possible explanation for this could be that SS has the lowest molecular weight, the lowest pK_a and the highest intrinsic solubility. When choosing a topical agent, its possibility to reach the effective dose in the pulp must be considered as well as the therapeutic effect and drug toxicity. However, there is no available information on the topical anti-inflammatory effect and toxicity of SS in the dental pulp. Further investigation into these issues is required.

MN, SS and NA were used in the present study. MN has been reported to be effective for the treatment of human carious dentine (Hoshino *et al.* 1989). SS is an NSAID and one of the components in a root canal sealer, Sealapex (Kerr, MI, USA). This sealer shows biological compatibility to the human fibroblast cell line (Willershausen *et al.* 2000). Additionally, SS inhibits the LPS-induced cyclo-oxygenase-2 (COX-2) activity in rats (Giuliano *et al.* 2001). NA, a propionic acid derivative, is also an NSAID, representing a new group of effective and useful aspirin-like agents. Topical application of NA is effective and safe enough for the treatment of acute soft tissue lesions (Baixauli *et al.* 1990). The results (Figs 2–4) suggest that these drugs may be suitable for iontophoretic delivery as they become ionized when dissolved in water.

The therapeutic utility of NSAIDs as anti-inflammatory drugs depends on their ability to inhibit COX-2. The IC_{50} , the concentration of NSAIDs that reduce COX-2 activity to 50% of a control value, is $100 \pm 16 \mu\text{g mL}^{-1}$ for SS and $1.3 \pm 0.8 \mu\text{g mL}^{-1}$ for NA in endotoxin-activated murine macrophages (Mitchell *et al.* 1993). The effective concentration of MN against obligate anaerobes is about $1 \mu\text{g mL}^{-1}$ (Ralph & Kirby 1975). The present results indicated that SS did not reach that concentration. On the other hand, the effective concentrations of MN and NA were achieved through intact dentine by iontophoresis within 20 and 15 min (Figs 2–4), respectively. The results confirmed that the iontophoretic drug delivery could enhance drug diffusion and shorten the time to reach the effective concentration. However, it might be more useful to use more potent drugs which could reach the effective concentration in the pulp promptly.

For caries-affected dentine, the effective concentrations of SS, MN and NA were not achieved, despite the fact that iontophoresis facilitated the drug diffusion

(Figs 2–4). The low permeability of caries-affected dentine might prevent drugs from reaching the effective concentration in the pulp. The concentration used in this study may have been too low. Additionally, the potencies of prostaglandin synthetase inhibitors differ in different tissues and also depend upon the method of assessment. However, there have been no available reports on those potencies in the human pulp tissue. Therefore, the effective concentration and potency of NSAIDs in the inflammatory human pulp tissues needs to be further elucidated.

Conclusions

Presence of dental caries may inhibit drug diffusion through dentinal tubules into the pulp. However, the iontophoretic technique could enhance the delivery of ionized drugs through both intact and caries-affected dentine.

Iontophoresis, therefore, could be a useful tool for clinical use because of its noninvasiveness, increase in local concentration of drugs and shortening the necessary time for drug application. Further *in vivo* studies are needed.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports Science and Technology (No. 13877322). We thank Associate Professor Chihiro Kobayashi, Pulp Biology and Endodontics, Department of Restorative Sciences, Tokyo Medical and Dental University, Japan, for his helpful advice on an electrical impedance measurement.

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